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بسم الله الرحمن الرحیم

Brucella vaccine

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Characteristics

- Brucella : Small, non-motile, non-spore forming, grow aerobically
- Some spp. Require supplemental carbon dioxide for primary isolation.
- Any high-quality peptone-based media enriched with blood or serum serve for in vitro cultivation.
- Isolation from clinical specimens require prolonged (≥ 30 days) incubation.
- Brucella strains always are catalse-positive; but oxidase and urease and H_2S production are vary.

Taxonomy:

- Bacteria

Proteobacteria

Alphaproteobacteria

Rhizobacteria

Brucellaceae

Brucella

Source: C Cox, The Wellcome Trust, Illustrated History of Tropical Diseases

Sir David Bruce (1855-1931)

- British Army physician and microbiologist
- Discovered *Micrococcus melitensis*

Professor FEG Cox, *The Wellcome Trust, Illustrated History of Tropical Diseases*

Bernhard Bang (1848-1932)

- Danish physician and veterinarian
- Discovered *Bacterium abortus* could infect cattle, horses, sheep, and goats

The Many Names of Brucellosis

Human Disease

- Malta Fever
- Undulant Fever
- Mediterranean Fever
- Rock Fever of Gibraltar
- Gastric Fever

Animal Disease

- Bang's Disease
- Enzootic Abortion
- Epizootic Abortion
- Slinking of Calves
- Ram Epididymitis
- Contagious Abortion

Occurrence in Wildlife











Epidemiology of Brucellosis

- Animals are natural reservoir (cattle, goats, sheep, swine,bison, elk, dogs, foxes, coyotes)
- 500,000 human cases per year worldwide
- Less than 100 annual cases in the U.S. due to successful control of the disease in livestock and the animal reservoir
- Transmission via :

  i) Ingestion of contaminated milk or cheese

  ii) Direct contact with infected animals or animal products

Epidemiology... (Cont.)

- Brucellosis is zoonosis , all infections, derive directly or indirectly from animals exposure.
- Disease exists world-wide, esp. Mediterranean, Arabic Peninsula, Indian subcontinent, parts of Mexico and Central South America.
- B. abortus found mainly in cattle, but others spp. like buffalo, camel can be affected.
- B. Melitensis primary affects goats and sheep. Camels can be important source in some countries.
- B. Suis biovars 1-3 in domestic and feral swine, cause human disease.

Epidemiology… (Cont.)

Routes of transmission to human include :

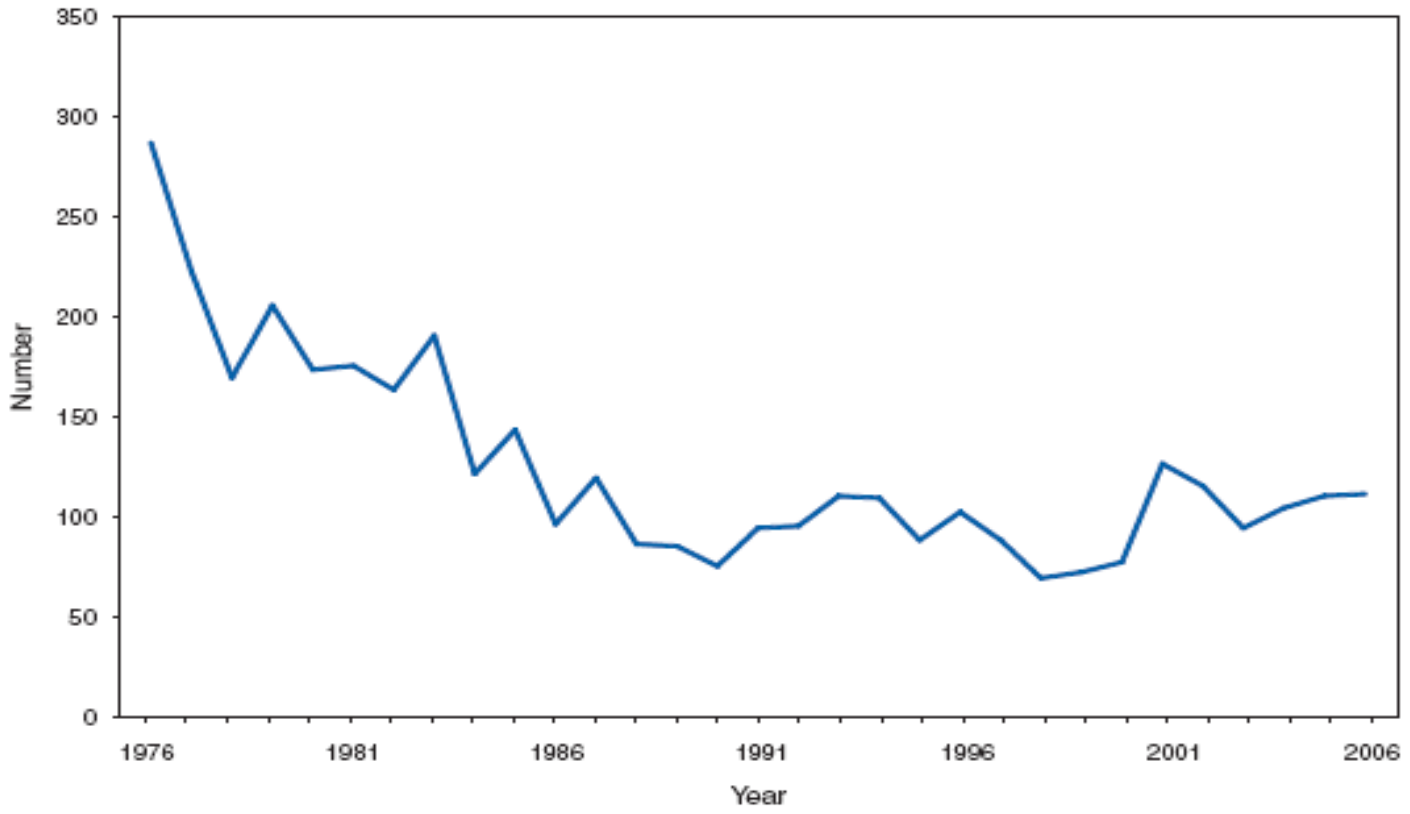
- Direct contact with animals or their secretions, through cuts and skin abrasions.
- Infected aerosols inhaled or inoculated into eye conjunctival sac.
- Ingestion of unpasteurized dairy products.

Epidemiology... (Cont.)

- **Meat products** are rare source of infection because meat is rarely eaten raw and organisms are in low number of muscle tissue.
- **Blood** and **bone marrow** may transmit disease when ingested in some cultures.
- **Human-to-human transmission** is unusual but rare cases suspected to be sexually transmitted.

Brucellosis in U.S. 1975-2006

BRUCELLOSIS. Number of reported cases, by year — United States, 1976–2006



The incidence of brucellosis has remained stable in recent years, reflecting an ongoing risk for infection with *Brucella melitensis* and *B. abortus* acquired through exposure to unpasteurized milk products in countries with endemic brucellosis in sheep, goats, and cattle and *B. suis* acquired through contact with feral swine in the United States.

Etiologic Agents

Brucella spp.	Source	Virulence	Infective Dose
B. melitensis	Goats, sheep, Cattle, Swine	++++	1 – 10
B. suis	Swine, Cattle	+++	1,000 – 10,000
B. abortus	Cattle	++	100,000
B. canis	Dogs	+	1,000,000

Resistance of brucella

Heating at 60ºC	For 10 minutes
Phenol 1%	For 15 minutes
Direct sunlight	In a few hours
Milk	For several days
Milk	(till the milk turns sour)
Fresh cheese	For 3 months
Tap-water	For 57 days
Human urine	For 1 week
Dust	For 6 weeks
Damp soil	For 10 weeks
Animal feces	For 100 days

Pathogenesis

- B. melitensis and B. suis, more virulent than B. abortus and B. canis.
- Infection with any B. species, including attenuated vaccine strains can cause serious human disease
- Disease determined by :
  - ✓ Host nutritional and immune status
  - ✓ Size of infectious inoculum
  - ✓ Route of transmission

  Ex : Low gastric juice PH, more effective in preventing B. abortus than B. melitensis infection when administered by oral route

A. Skin abrasion, conjunctivae, inhalation or ingestion are paths of infection
B. Bacteria engulfed by neutrophils and monocytes
C. Localize in regional lymph nodes
D. Infect phagocytic cells in the RE system and form granulomas

Pathogenicity and Virulence

- In the preferred host, localization also occurs in the tissues of the genital tract and in the mammary glands. In the case of B. abortus, B melitensis, and B. suis, this has been attributed to the presence of isoerythritol in the target tissues of cattle, sheep, goats, and pigs

- Abortion is a frequent consequence of infection in the pregnant female, and orchitis and epididymitis can result in the male

Pathogenicity and… (Cont.)

- Four major virulence determinants are LPS, type-4 secretion system (T4SS), BvrR/BvrS
- Studies have shown that Brucella OPS (O polysaccharide) is involved in cellular invasion and in the survival of the organism
- Seems as a stealth pathogen, penetrating host cell without activating antimicrobial mechanisms
- Once engulfed, the microorganisms release undetermined factors from the T4SS as well as express periplasmic cyclic β-1,2-glucan and OPS to prevent fusion of the Brucella-containing vacuole(BCV) with lysosomes

Dissemination

- After phagocytosis, brucellae probably multiply in the **lymph nodes**, Subsequently, as the bacteria are released from the dying cells that they have parasitized, they enter the **blood** and produce the **bacteremia** that normally accompanies the acute febrile phase of the diseas, From the blood, the organisms are distributed throughout the **reticuloendothelial system** and may be present in large numbers in the liver and spleen. They may also localize in many other sites including the **joints, heart, kidneys, central nervous system (CNS),** and **genital tract**

Antigenic Structure

LPS

- A , M Antigenic epitopes associated with the LPS complexes

OMPs

- Outer-membrane proteins include the omp of groups 1,2, and 3

Intracellular Antigens

- Released on breakage of Brucella cells , for the most part, proteins, glycoproteins, and peptides, The antigens designated A1, A2, A3, A4, B1, B2 and C, An 18 kDa cytoplasmic protein has been described as an indicator of active infection in cattle and humans

Species	Biogroup	CO_2 requirement	H_2S production	Growth in presence of 1 in 50 000 (20 µg/ml)		Agglutination by monospecific serum[b]			Preferred natural host	Remarks
				Thionin	Basic fuchsin	A	M	R		
B. melitensis	1	–	–	+	+	–	+	–	Sheep, goat	Typical melitensis
	2	–	–	+	+	+	–	–	Sheep, goat	
	3	–	–	+	+	+	+	–	Sheep, goat	
B. abortus	1	(+)	+	–	+	+	–	–	Cattle	Typical abortus
	2	(+)	+	–	–	+	–	–	Cattle	Wilson II type
	3	(+)	+	+[c]	+	+	–	–	Cattle	Rhodesian type
	4	(+)	+	–	(+)	–	+	–	Cattle	
	5	–	–	+	+	–	+	–	Cattle	British 'melitensis'
	6	–	–	+[c]	+	+	–	–	Cattle	
	9	–	+	+	+	–	+	–	Cattle	
B. suis	1	–	+	+	(–)	+	–	–	Pig	American suis
	2	–	–	+	–	+	–	–	Pig, hare	Danish suis
	3	–	–	+	+	+	–	–	Pig	American 'melitensis'
	4	–	–	+	(–)	+	+	–	Reindeer	'*B. rangiferi tarandi*'
	5	–	–	+	–	–	+	–	Rodents	Caucasus only
B. neotomae		–	+	–	–	+	–	–	Desert wood rat	USA only
B. canis		–	–	+	(–)	–	–	+	Dog	Canine abortion organism
B. ovis		+	–	+	(–)	–	–	+	Sheep	Ram epididymitis organism
B. 'maris'		+ or –	–	+	+	+	+ or –	–	Marine mammals	Pathogenicity unknown

+, positive; –, negative; (+), usually positive; (–), usually negative.

a) For oxidative metabolism, see Table 66.2; for phage susceptibility, see Table 66.4.

b) A, abortus; M, melitensis; R, rough.

c) *B. abortus*, biogroup 3 grows in the presence of 1 in 25 000 thionin; biogroup 6 does not. Former *B. abortus* biogroups 7 and 8 are no longer regarded as valid.

Human congenitally infection

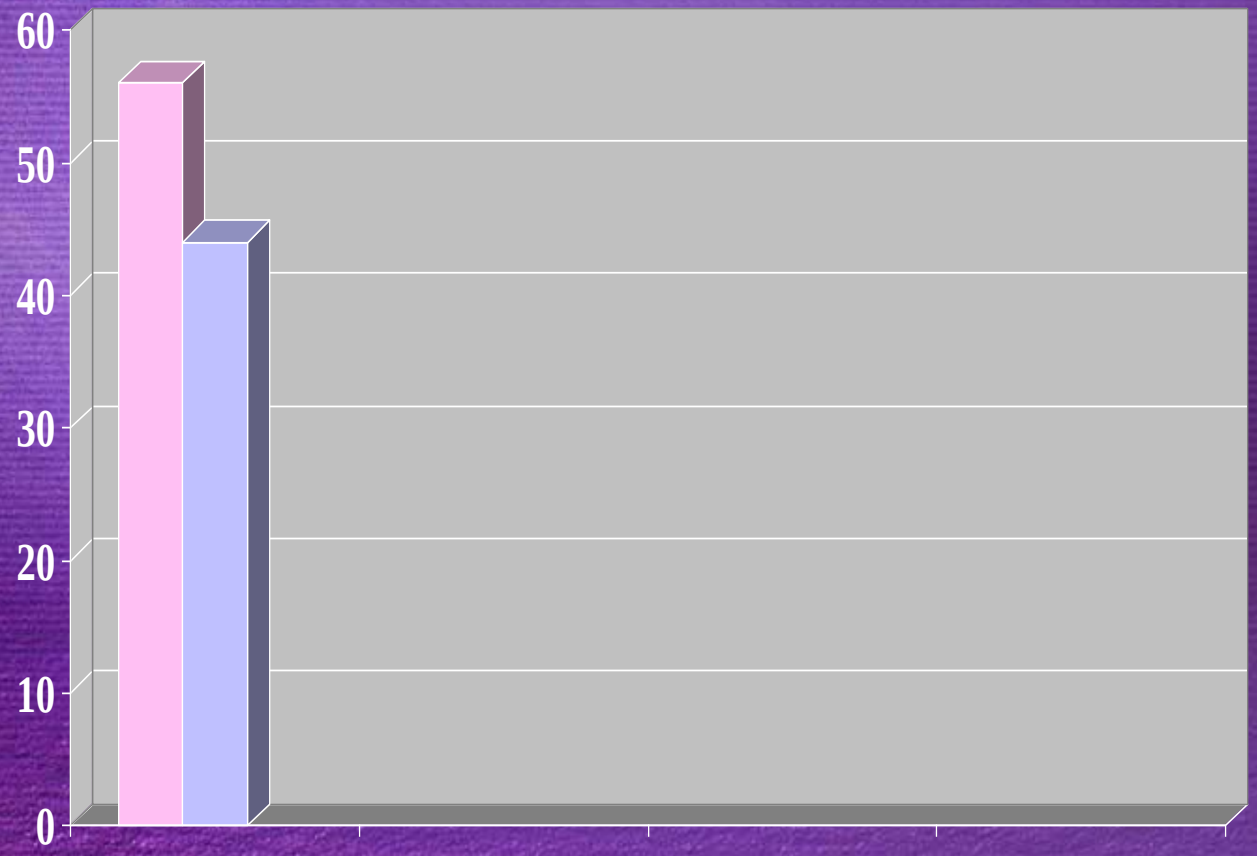
- **Congenitally infected infants**
  - Low birth weight
  - Failure to thrive
  - Jaundice
  - Hepatomegaly
  - Splenomegaly
  - Respiratory difficulty
  - General signs of sepsis (fever, vomiting)
  - Asymptomatic

Prognosis of disease in Human

- May last days, months, or years
- Recovery is common
- Disability is often pronounced
- About 5% of treated cases relapse
- Case-fatality rate : <2% (untreated)
  - Endocarditis caused by *B. melitensis*

Effects of Gender and Age

- **Men** aged 15-45 years are affected twice as often as women of the same age
- **In Iran** both sexes are nearly always equally contacted with contaminated animals and dairy products and there is not a significant predominance of male to female brucellosis
- **Children** are affected very much less than adults, The odd low incidence in children is unexplained, It may be that **gastric acidity** is less often deficient than in adult



Male
56%

Female
44%

Sex distribution in Iran 1370

Brucellosis in animals

- *Brucella* infect organs rich in erythritol (a sugar metabolized in preference to glucose) like breast, uterus, placenta and epididymis
- Asymptomatic carriage, sterility or abortions

- Transmitted by contact with infected tissue or milk (oral, aerosol or abrasion)

Transmission of brucellosis

- Farmers / Ranchers
- Veterinarians (food animal practice)
- Brucellosis eradication personnel
- Meat inspection personnel
- Abattoir workers
- Laboratory personnel

Reservoirs

- Mammalian parasite
- Host specificity is not exclusive
- **Horse** is occasionally infected
- Serum from **camels** have proved positive
- Have been isolated from **foxes, buffaloes, elk, moose, deer, polecats**
- **Rabbits** have been infected
- **Flies, mosquitoes, ticks** and other insects can be infected with all species
- Ticks have transmitted infection to guinea pigs by biting
- Dogs and cats are fairly resistant
- Has not been found in monkeys

Species	Biovar/Serovar	Natural Host	Human Pathogen
B. abortus	1-9	cattle	yes
B.melitensis	1-3	goats, sheep	yes
B. suis	1-3	swine	yes
	2	hares	yes
	4	reindeer, caribou	yes
	5	rodents	yes
B. canis	none	dogs, other canids	yes
B. ovis	none	sheep	no
B. neotomae	none	Desert wood rat	no
B. maris (?) *B.Pinnipediae* *B.cetaceae*	?	marine mammals	yes

Clinical Presentation of Human Brucellosis

- Acute disease often develops with initial nonspecific symptoms of malaise, chills, fatigue, weakness, myalgias (muscles), weight loss, arthralgias (joint), and nonproductive cough
- Mild disease with rare suppurative complications
- Chronic disease and recurrence are common because it can survive in phagocytic cells and multiply to high concentrations

Control & Prevention of Brucellosis

- In 1934, the U.S. Department of Agriculture (USDA) established the National Brucellosis Eradication Effort which is managed by Animal, Plant, and Health Inspection Service (APHIS)
- APHIS certifies states as brucellosis-free, classes A, B, or C of which all states are currently classified A
- Serology & confirmatory bacterial culture to identify infected animals
- Positive animals/herds are destroyed
- Vaccination is available but is not a 100% effective and is costly to cattle ranchers

Host Immunity

- Wright , 2ME test shown to be useful to monitor Brucellosis course and response to therapy.
- Application of enzyme-linked immunoabsorbent assay (ELISA) measures immune response to therapy.
- IgM antibodies appear within 1st week of infection and followed by switch to IgG synthesis after 2nd week.

Complications

Nervous System

- Direct CNS invasion by brucellosis is less than 5%
- CNS Complications :
  - Meningitis
  - Encephalitis
  - Myelitis , radiculoneuritis, Brain abscess
  - Demyelinating syndromes
  - Meningovascular syndromes
  - Acute and chronic meningitis are Most frequent CNS complication, and can be occur in late course
  - Brucella meningitis difficult to distinguish from other causes of meningitis
- CSF: Lymphocytic pleocytosis, elevated protein, low to normal glucose
- Gram stain usually negative
- Culture positive <25% of cases

GIT

- **Pathologic lesions:**
  - Intestinal mucosa hyperemia with peyer's patches inflammation.
  - Acute ileitis radiologically and histologically in patients with B. melitensis colitis

Hepatobiliary system (HPS)

- Liver is a largest reticuloendothelial organ that probably always involved in brucellosis.
- LFT, usually slightly elevated.

❑ B. abortus infection:

✓ Granulomas indistinguishable from sarcoidosis.

❑ B. melitensis infection :

✓ Lesions resembling viral hepatitis

▪ Suppurative abscess of liver and spleen common with B. suis infection and occasionally with B. melitensis

▪ Brucella rarely causes acute cholecystitis, pancreatitis and spontaneous bacterial peritonitis (SBP)

Hematologic Complications

- Hematologic manifestations of brucellosis include anemia, leukopenia, thrombocytopenia and clotting disorders
- Granulomas found in B. marrow in up to 75% of cases, but they are small and indistinct
- Severe thrombocytopenia with cutaneous purpura reported

Cutaneous Complications

- Cutaneous lesions occur in 5% of patients with brucellosis
- Many transient, nonspecific lesions described, including rashes, papules, ulcers, petechiae, purpura, and vasculitis

Others

- Osteoarticular complications reported in 20-60% of pts. infected with brucellosis.
- Bone and joint lesions include:
  - Arthritis
  - Spondylitis
  - Osteomyelitis
  - Bursitis
  - Sacroiliitis most commonly reported complication.

Synovial fluid analysis reveal brucellae in about 50% of cases

Endocarditis occurs in less than 2% of cases, but accounts for majority of brucellosis-related deaths.

- Before effective therapy, including valve replacement surgery, Brucella endocarditis is nearly always fatal.

Others (cont.)

Orchitis occurs in up 20% of men with brucellosis.

In women, rare cases salpingitis, cervicitis and pelvic abscess reported.

- Principal brucellosis in animals is spontaneous abortion and presence of erythritol in tissues of susceptible animals, thought to play role in localization of brucellae in genital tract.

- Brucellosis can result in human abortions, but unclear whether it is more frequent than with other bacteremic infections.

Diagnosis

- Because signs and symptoms of brucellosis are nonspecific, it is important to **o**btain detailed history including occupation, exposure to animals, travel to enzootic areas, and ingestion of high-risk foods, as unpasteurized dairy products
- WBC : normal or low and may not suggests infectious process
- Anemia, leukopenia, and thrombocytopenia are common findings
- E.S.R. variable and of little diagnostic value
- Bone marrow cultures have higher yield than blood.

Diagnosis

- Bacterial Isolation
- Serological Test
- Molcular Assay

Culture Media

- Serum Dextrose Agar (SDA)
- Glycerol Dextrose Agar (GDA)
- Brucella Broth
- Brucella Agar
- Modified Farrell’ s Medium (SDA + Antibiotics)
- Brodie and Sinton Liquid Medium
- Castaneda
- Modified Thayer Martin Medium
- TSB

Castaneda Media

Diagnosis

Serological Test

- Brucella “ELISA” can be definitive, when agglutination tests equivocal
- Rose Bengal test
- Wright test
- 2 Me

Coombs Wright

- Ring Test is used to suspected cattle herd in animals

Molcular Assay

- Conventional PCR
- Multi plex PCR
- Real Time PCR
- VNTR PCR

Determination of biovars

depends on :

- Need to CO2
- Production of H2S
- Agglutination with anti A or anti M
- Sensitivity or resistance to Thionine and Fuschine
- Phage typing

Typing of Brucella

- Culture in Brucella agar
- CO_2 requirement
- H_2S production
- Agglutination with A,M monospecific antiserum
- Acryflavin test
- Culture with tionin and fuschin dye
- Phage typing

Brucella as a Biological Weapon

- Aerosolized *B. melitensis*
  - City of 100,000 people
  - Inhale 1,000 cells
  - Case-fatality rate of 0.5%
  - 50% hospitalized for 7 days
    - 5% relapsed
- Results
  - 82,500 cases requiring extended therapy
  - 413 deaths
  - $477.7 million economic impact

Treatment

- **Tetracyclines** are most active drugs for treating brucellosis, because of high relapse rate with single-drug therapy, combination therapy is recommended.
- Many studies showed **tetracycline combined with streptomycin** for three weeks is the most effective treatment.
- **Doxycycline + Streptomycin** is more effective than **Doxycycline + Rifampicin**, especially for patients with complications such as spondylitis
- **Cotrimoxazole** + **Aminoglycoside** reported to be successful in treating children younger

Prevention

- Prevention of human brucellosis depend on :

  Control and elimination of Brucellosis in domestic animals

- Effective attenuated live bacterial vaccines exist for :
  - B. abortus (**Strain 19 / RB51**)
  - B. melitensis (**Strain Rev-1**)
  - No vaccines for B. suis and B. canis
- On rare occasions, accidents with **S19** and **Rev-1** caused human brucellosis.
- RB51 (stable rough mutant) largely replaced strain 19 as preferred bovine vaccine (**USA / IRAN**).
- Strain RB51, has advantage of protecting cattle without inducing antibody response, and RB51 appears to lack virulence for humans and despite accidents, no proven cases of human RB51 infection.
- No licensed human vaccine is available

Hindawi Publishing Corporation
BioMed Research International
Volume 2013, Article ID 743509, 8 pages
http://dx.doi.org/10.1155/2013/743509

Delelopment Of Brucella Vaccines

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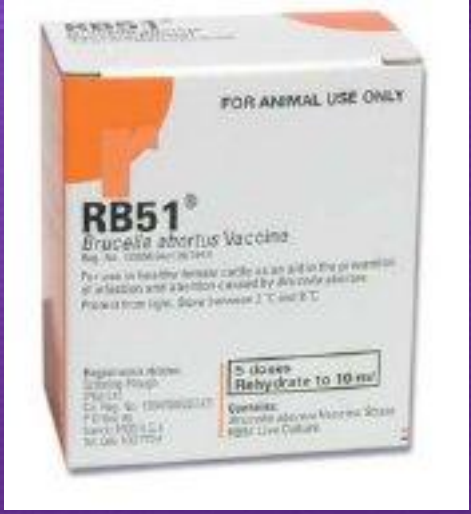
Received 11 January 2013; Accepted 9 May 2013

Academic Editor: Adolfo Paz Silva

Brucellosis is a worldwide zoonosis affecting animal and human health. In the last several decades, much research has been performed to develop safer *Brucella* vaccines to control the disease mainly in animals. Till now, no effective human vaccine is available. The aim of this paper is to review and discuss the importance of methodologies used to develop *Brucella* vaccines in pursuing this challenge.



Indications:

For use in healthy female cattle as an aid in the prevention of infection and abortion caused by *Brucella abortus*.

Storage Instructions:

Protect from light. Store between 2°C and 8°C.

Composition:

This lyophilised vaccine contains the RB51 strain of *Brucella abortus.*

Warnings

Vaccination of pregnant animals may cause abortion

Evaluation and Importance of WboA gene in RB51 brucella Vaccine

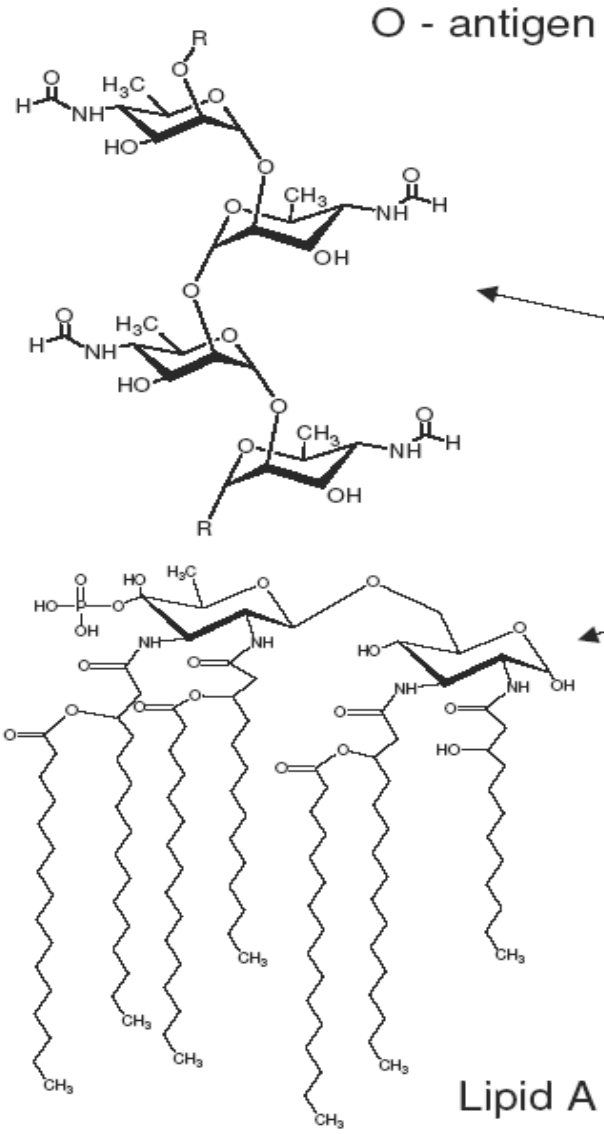
- Brucella spp. are Without classic virulen like toxin and capsule

- Lps:

key molecule in virulence and intracellular multiplication

Lps structure

- Lipid A
- Core
- O antigen



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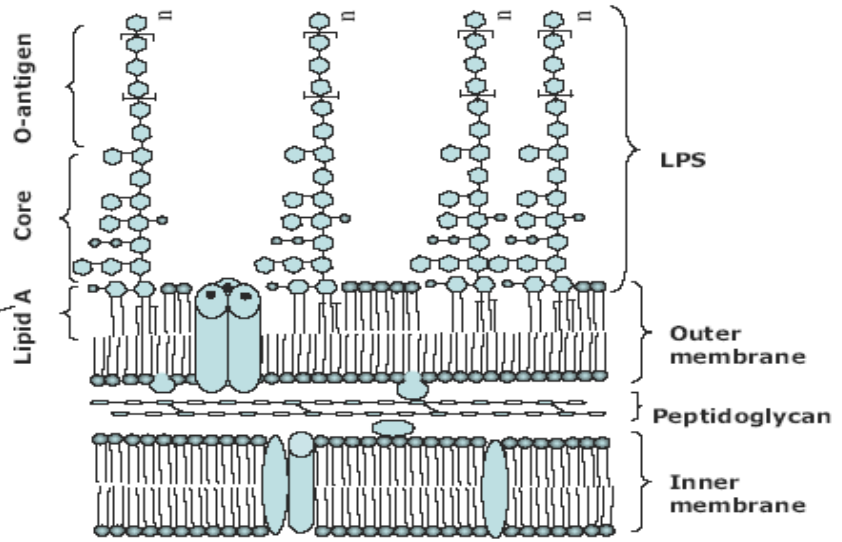

Lps structure

Genes involved in Lps biosynthesis:

- Prosamine synthetase (per)
- Phosphomannomutase (pmm or manB)
- Phosphoglucomutase(pgm)
- ABC type transportase(Wzm and Wzt)
- Mannosyltransferase(wbkA.wboA)

WboA

- Location: chromosome I
- Length: 1233 bp
- Encode: glycosyltransferase
- Function: O side chain biosynthesis

Smooth strain (virulent)

(LPS contain O side chain)

Rough strain (non virulent)

(LPS without O side chain)

Brucella abortus strain 2308

(smooth=Virulent)

RB51

Rough=Vaccine

WboA (Brucella abortus strain2308)

RB51

RB51WboA

No change in virulence and phenotype

but

RB51 **(1.5 unit of immunity)**

RB51WboA **(4 unit of immunity)**

- IRIBA Brucella Vaccine is a live attenuated rough strain similar to Rb51 vaccine that produce in Razi Vaccine and Serum Research Institute.
- Full Dose
- Reduced Dose


Thank You